



## **Peptide receptor radionuclide therapy in gastroenteropancreatic NEN G3 a multicenter cohort study**

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# 1 **Peptide Receptor Radionuclide Therapy in Gastroenteropancreatic**

## 2 **NEN G3: a multicenter cohort study**

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36

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38

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40 High-grade; Peptide receptor radionuclide therapy; Radiolabeled somatostatin analogues;

41 <sup>177</sup>Lutetium; <sup>90</sup>Yttrium; Progression-free survival; Overall survival.

42

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45

46   **Abstract**

47   Peptide receptor radionuclide therapy (PRRT) is an established treatment of metastatic  
48   neuroendocrine tumors grade 1-2 (G1-G2). However, its possible benefit in high-grade  
49   gastroenteropancreatic (GEP) neuroendocrine neoplasms (NEN G3) is unknown. We therefore  
50   aimed to assess the benefits and side effects of PRRT in patients with GEP NEN G3. We performed  
51   a retrospective cohort study at 12 centers to assess efficacy and toxicity of PRRT in patients with  
52   GEP NEN G3. Outcomes were response rate, disease control rate, progression-free survival (PFS),  
53   overall survival (OS) and toxicity. We included 149 patients (primary tumor: pancreatic n=89,  
54   gastrointestinal n=34, unknown n=26). PRRT was 1st-line (n=30), 2nd-line (n=62) or later line  
55   treatment (n=57). Of 114 patients evaluable, 1% had complete response, 41% partial response, 38%  
56   stable disease and 20% progressive disease. Of 104 patients with documented progressive disease  
57   before PRRT, disease control rate was 69%. The total cohort had median PFS of 14 months and OS  
58   29 months. Ki-67 21-54% (n=125) vs. Ki-67 $\geq$ 55% (n=23): PFS 16 vs. 6 months (p<0.001) and OS  
59   31 vs. 9 months (p<0.001). Well (n=60) vs. poorly-differentiated NEN (n=62): PFS 19 vs. 8 months  
60   (p<0.001) and OS 44 vs. 19 months (p<0.001). Grade 3-4 hematological or renal toxicity occurred in  
61   17% of patients. This large multicenter cohort of patients with GEP NEN G3 treated with PRRT  
62   demonstrates promising response rates, disease control rates, PFS and OS as well as toxicity in  
63   patients with mainly progressive disease. Based on these results, PRRT may be considered for  
64   patients with GEP NEN G3.

## 69    **Introduction**

70    Neuroendocrine neoplasms (NEN) are a very heterogeneous entity classified according to primary  
71    tumor location, stage, proliferation rate and differentiation. The 2010 World Health Organization  
72    (WHO) Classification grades NEN according to the proliferation index Ki-67;  $\leq 2\%$  (Grade 1, G1),  
73    3-20% (G2) and  $> 20\%$  (G3) (Bosman, et al. 2010). G1-G2 were collectively referred to as  
74    neuroendocrine tumors (NET) and G3 as neuroendocrine carcinoma (NEC). The classification is  
75    strongly prognostic, but is also used to guide treatment decisions. In 2017, WHO refined the  
76    classification of pancreatic NEN; G3 tumors are further classified as well (NET G3) and poorly  
77    differentiated (NEC) (Kloppel, et al. 2017), and a similar expansion to gastrointestinal (GI) G3  
78    tumors is anticipated in the next WHO classification. The NET category is now only used for well-  
79    differentiated tumors regardless of their proliferation index (G1-G3), whereas the NEC category is  
80    used for poorly differentiated high-grade neuroendocrine carcinomas (G3). The terminology of  
81    NEN G3 relates to all high-grade (G3, Ki-67  $>20\%$ ) neuroendocrine malignancies; i.e. both NET  
82    G3 and NEC.

83                    Gastroenteropancreatic (GEP) NENs G3 are rare, highly malignant, with poor  
84    prognosis and limited therapeutic options (Garcia-Carbonero, et al. 2016; Ilett, et al. 2015; Sorbye,  
85    et al. 2014). The majority of patients have metastases at the time of diagnosis and median overall  
86    survival (OS) is less than 6 months including all patients (Dasari, et al. 2018). Platinum-based  
87    chemotherapy is the standard treatment in metastatic disease with response rates of 30-35%,  
88    progression-free survival (PFS) of 4-5 months and OS 11-14 months (Heetfeld, et al. 2015; Sorbye,  
89    et al. 2013; Walter, et al. 2017; Yamaguchi, et al. 2014).

90                    In metastatic GEP NET G1-G2, peptide receptor radionuclide therapy (PRRT)  
91    targeting somatostatin receptors has been used with excellent results for the last two decades in

92 Europe and Israel (Bodei, et al. 2011; Imhof, et al. 2011; Kwekkeboom, et al. 2008; Pfeifer, et al.  
93 2011; Romer, et al. 2014). The recent NETTER-1 phase 3 trial of patients with somatostatin  
94 receptor imaging (SRI) positive NET G1/G2 supports this approach (Strosberg, et al. 2017). In  
95 contrast, PRRT has generally not been recommended for GEP NEN G3 based on expectance of low  
96 expression of somatostatin receptors and rapid growth behavior. According to guidelines, PRRT can  
97 be considered in SRI-positive NET G3, but data are lacking (Garcia-Carbonero et al. 2016). PRRT  
98 could, however, be a relevant therapeutic option for NEN G3 since SRI positivity has been reported  
99 for both NET G3 and NEC (Heetfeld et al. 2015; Raj, et al. 2017; Sorbye et al. 2013; Velayoudom-  
100 Cephise, et al. 2013), as well as having expression of somatostatin receptor 2A on  
101 immunohistochemistry (Konukiewicz, et al. 2017).

102 Randomized large studies to assess the benefit of specific treatments are often not  
103 feasible to perform in very rare diseases. Large retrospective datasets may then initially be the only  
104 way on which to base treatment decisions. In a large multicenter international cooperation, we  
105 therefore collected retrospectively the outcomes after PRRT in patients with GEP NEN G3.

106

## 107 **Methods**

### 108 **Patients**

109 At 12 university hospitals, we retrospectively included patients that fulfilled the following criteria:  
110 1) GEP NEN or NEN of unknown primary with dominance of abdominal metastases, 2) Ki-67 >  
111 20%, and 3) treated with PRRT. Data on demographics, diagnosis, previous treatments, PRRT,  
112 outcome and toxicity were registered. SRI (<sup>68</sup>Ga-somatostatin analogue positron emission  
113 tomography [PET]/computer tomography [CT], or <sup>111</sup>In-octreotide or <sup>99m</sup>Tc-tektrotyd scintigraphy)  
114 results were reported as tumor uptake in relation to liver uptake (none, < liver, = liver or > liver)

115 and used as a surrogate for somatostatin receptor density. <sup>18</sup>F-Flour-Deoxy-Glucose (FDG) PET/CT  
116 results were reported as tumor uptake present or not (positive or negative by qualitative  
117 assessment). Histological examination included chromogranin A (CgA) and synaptophysin staining,  
118 Ki-67% in hot-spots, and tumor differentiation (poor, intermediate and well). Most of the centers  
119 have specific NET pathologists and in cases where differentiation was lacking in the original  
120 pathology report, a reclassification was done if sections were available. Plasma values of  
121 chromogranin A, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) obtained at the time  
122 of first PRRT were reported.

123               Patients were grouped according to Ki-67 index (21-54% and  $\geq 55\%$ ) based on the  
124 Nordic NEC study and other reports (Garcia-Carbonero et al. 2016; Sorbye, et al. 2018; Sorbye et  
125 al. 2013; Thang, et al. 2018). Furthermore, patients were grouped by combined Ki-67% and  
126 differentiation: Ki-67: 21-54% and well-differentiated tumor (NET G3) vs. Ki-67: 21-54% and  
127 poorly differentiated tumor (NEC; Ki-67 21-54%) vs. Ki-67  $\geq 55\%$  and poorly differentiated tumors  
128 (NEC; Ki-67  $\geq 55\%$ ) (Milione, et al. 2017).

129               Ethical committee approval was obtained in accordance with regional guidelines  
130 (either approval of the study or exempt of application due to the retrospective design). Regional  
131 ethics committees for participating centers are Rigshospitalet (Videnskabsetisk Komité, Region  
132 Hovedstaden) and Aarhus University Hospital (Videnskabsetisk Komité, Region Midt), Denmark;  
133 University Hospital Bonn (Ethikkommission an der Medizinischen Fakultät der  
134 RheinischenFriedrich-Wilhelms-Universität Bonn) and University Hospital Gießen and Marburg  
135 (Ethics Committee of the Philipps-University Marburg, Medicine), Germany; Hadassah-Hebrew  
136 University Medical Center (Hadassah-Hebrew University Medical Center Institutional Ethical  
137 Committee), Israel; European Institute of Oncology (Ethics Committee), Italy; Erasmus Medical  
138 Center (Medical Research and Ethics Committee, Rotterdam), The Netherlands; MSWiA Hospital



139 Warsaw (Komisja Etyki i Nadzoru nad Badaniami na Ludziach), Poland; Uppsala University  
140 Hospital (Uppsala Regionala Etikprövningsnämnden), Sweden; University Hospital Basel  
141 (Ethikkommission beider Basel), Switzerland; Churchill Hospital (Oxford Research and Ethics  
142 Committee) and Imperial College London (Regional Ethics Committee of Wales), United Kingdom.  
143 Patients gave informed consent before receiving PRRT.

144

## 145 **Treatment**

146 Patients received PRRT according to local guidelines at their respective institution. In general,  
147 treatment was given intravenously and consisted of a radioisotope (<sup>177</sup>Lutetium, <sup>90</sup>Yttrium or <sup>111</sup>  
148 Indium) conjugated with a somatostatin analogue (octreotide or octreotate). Patients were planned  
149 to a series of PRRT, typically consisting of four cycles each and separated by approximately 8  
150 weeks. The intended cumulative activity was calculated by taking renal function and bone marrow  
151 irradiation into account. To reduce renal irradiation, patients were pretreated with an intravenous  
152 amino-acid solution. Planned PRRT cycles were discontinued in case of progression of disease or  
153 adverse effects limiting further cycles.

154

## 155 **Outcomes**

156 Response rate (RR) was defined as complete response (CR) or partial response (PR) according to  
157 the response evaluation criteria in solid tumors (RECIST 1.1) (Eisenhauer, et al. 2009). Disease  
158 control rate (DCR) was defined as CR or PR in all patients or stable disease (SD) in patients with  
159 progressive disease (PD) at the start of PRRT. PFS was time from first cycle of PRRT to disease  
160 progression radiologically by RECIST 1.1, or clinically assessed by a physician [i.e. worsening of  
161 performance status due to NEN]. If no progression was documented, date of death or date last

162 follow-up if alive was used. OS was time from first cycle of PRRT to death or date of last follow-up  
163 if still alive. Toxicity was reported as acute if occurring during PRRT and as long-term if occurring  
164 after PRRT and within 1 year of PRRT. Toxicity was graded according to the Common  
165 Terminology Criteria for Adverse Events v.4, reporting grade 3-4 only.

166

## 167 **Statistics**

168 Continuous variables are reported as median and range. By means of Kaplan-Meier estimation, PFS  
169 and OS was calculated and reported as median with 95 % confidence intervals (CI). Log-rank test  
170 was used to compare PFS and OS estimates between groups. Cox regression analysis was  
171 performed for PFS and OS with covariates: age, gender, performance status, SRI tumor uptake, Ki-  
172 67 (dichotomized), primary tumor site, tumor morphology (well vs. poorly differentiated, excluding  
173 the intermediate group due to few cases), plasma LDH and plasma ALP. P-values < 0.05 were  
174 considered statistically significant. All analyses were performed using SPSS statistics 25.

175

## 176 **Results**

### 177 **Patients**

178 From August 1999 to May 2017, 149 patients with GEP NEN G3 received PRRT at 12 centers  
179 (Table 1). The primary tumor site was predominantly in the pancreas (n=89) or unknown (n=26).  
180 Other sites included the esophagus (n=2), stomach (n=4), gallbladder/common bile duct (n=2),  
181 small bowel (n=18), colon (n=3), rectum (n=3) and other abdominal sites (n=2), here collectively  
182 referred to as GI (n=34). All but two patients had metastatic disease. The median Ki-67 was 30%,  
183 ranging from 21-100%. Ki-67 21-54% was found in the majority of patients (n=125) vs.  $\geq 55\%$

184 (n=23), missing for one patient. Tumor morphology was equally distributed among poorly (n=62)  
185 and well differentiated (n=60) with only few cases of intermediate differentiation classification  
186 (n=9). Seventeen of 20 patients (85%) with Ki-67%  $\geq$  55% vs. 44 of 110 patients (40%) with Ki-67  
187 21-54% had poorly differentiated tumor morphology. All patients with SRI showed tumor uptake,  
188 predominantly > liver uptake.

189

## 190 **Treatment**

191 At the start of PRRT, 104 patients (70%) had radiologically progressive disease (determined by  
192 RECIST in 67 patients), which also was the main indication for PRRT (65%) (Table 2). The median  
193 time from diagnosis to first PRRT was 8 months (range 0-174). PRRT was frequently given as 2nd-  
194 line (n=62) or a later line of treatment (n=57). Patients received a median of 4 cycles PRRT (range  
195 1-15) with a median cumulative activity of 18 gigabecquerel (range 4-85). Radioisotopes  
196 <sup>177</sup>Lutetium and/or <sup>90</sup>Yttrium were used for PRRT in all patients other than a single patient who  
197 received <sup>111</sup>Indium. Concurrent chemotherapy was applied for six patients. Overall, 98 patients  
198 (65.8%) completed their planned protocol of PRRT cycles, while 51 patients did not (Table 2). The  
199 main reasons for not completing the planned PRRT cycles were progressive disease (n=19), clinical  
200 deterioration (n=6) or toxicity (n=6). Data on treatment after PRRT was available for 118 patients  
201 (79.2%). Chemotherapy (n=65) and somatostatin analogs (n=67) were frequently used, while  
202 surgery on the primary tumor or metastases (n=8), liver embolization (n=12) and external  
203 radiotherapy (n=19) were less frequently used.

204

## 205 **Response and survival analysis**

206 Of 114 patients evaluable by RECIST, 1 (1%) had CR, 47 (41%) PR, 43 (38%) SD and 23 (20%)  
 207 PD. An example of a PR is shown in Figure 1. Disease control was seen in 79 patients (69%)  
 208 responding to PRRT. RR did not differ among subgroups, including differentiation (42% vs. 43%  
 209 for well and poorly differentiated, respectively) and Ki-67 index (42% vs. 43% for Ki-67 21-54%  
 210 and Ki-67  $\geq 55\%$ , respectively) (Table 3). We observed similar RR with use of  $^{177}\text{Lu}$  or  $^{90}\text{Y}$  PRRT  
 211 and for patients from the 12 centers (data not shown). Median follow-up was 23 months (range 0-  
 212 210) and during follow-up 107 patients died. The cause of death was NEN in 91 of 94 cases with  
 213 available data. The median PFS was 14 months (95%CI 10.4-17.6) and median OS was 29 months  
 214 (95%CI 23.3-34.7) for all patients. Median PFS and OS were significantly longer for patients with a  
 215 Ki-67 21-54% ( $p < 0.001$ ), well differentiated tumor ( $p < 0.001$ ), PS  $< 2$  ( $p < 0.001$ ), normal plasma  
 216 levels of LDH ( $p < 0.001$ ) and ALP ( $p < 0.001$ ) (Figures 2-3). PFS and OS were independent of the  
 217 amount of SRI tumor uptake, primary tumor site and line of treatment. In univariate analyses of PFS  
 218 and OS, Ki-67 index, differentiation, PS as well as plasma LDH and ALP were statistically  
 219 significant predictors (Table 4). In multivariate analysis ( $n=75$ ), PS, plasma LDH and ALP were  
 220 statistically significant predictors for PFS and OS, and age was significant for PFS and  
 221 differentiation for OS (Table 5). Excluding plasma LDH and ALP from the multivariate analysis  
 222 resulted in 106 patients in the model; differentiation and PS were statistically significant predictors  
 223 for PFS and OS (data not shown).

224

## 225 **Toxicity**

226 Acute grade 3-4 toxicity occurred in 19 patients (13%), most frequently hematological ( $n=9$ ) or  
 227 renal ( $n=3$ ) (Table 2). In four patients, the acute hematological toxicity persisted beyond the time of  
 228 PRRT and was thus included as long-term toxicity as well. Another 15 patients without any acute

229 severe toxicity developed long-term hematological (n=11), renal (n=3) or not specified (n=1) grade  
230 3-4 toxicity. For first, second and later line of treatment 5 (17%), 16 (26%) and 13 (23%) patients  
231 had grade 3-4 toxicity, respectively. With  $^{177}\text{Lu}$  24 (24%),  $^{90}\text{Y}$  7 (21%) and combined  $^{177}\text{Lu}/^{90}\text{Y}$  3  
232 (25%) patients had grade 3-4 toxicity, respectively. Renal grade 3-4 toxicity occurred in two  
233 patients (6%) treated with  $^{90}\text{Y}$  and four patients (4%) treated with  $^{177}\text{Lu}$ .

234

## 235 **Discussion**

236 To the best of our knowledge, this is the largest study to assess the outcome after PRRT in patients  
237 with advanced high-grade GEP NEN. The majority of the patients had radiological progressive  
238 disease at the start of PRRT; RR was 42% and DCR was 69% for evaluable patients. A promising  
239 median PFS of 14 months and median OS of 29 months was found. Hematological or renal grade-3-  
240 4 toxicity occurred in 17% of patients, not more than observed for other patient groups given PRRT.  
241 These results suggest that PRRT can be effective and tolerable in high-grade GEP NEN patients.

242

## 243 **Comparison with standard treatment**

244 The current recommendations for first-line treatment of advanced GEP NEC is systemic platinum-  
245 based chemotherapy giving a RR of 30%, PFS 4-5 months and OS 11 months (Heetfeld et al. 2015;  
246 Sorbye et al. 2013; Walter et al. 2017; Yamaguchi et al. 2014). Second-line treatment for NEC is  
247 usually of short benefit with an estimated PFS of 3-4 months (Hadoux, et al. 2015; Hentic, et al.  
248 2012; Olsen, et al. 2014; Olsen, et al. 2012; Walter et al. 2017; Welin, et al. 2011). The Nordic NEC  
249 study showed a poorer RR to platinum-based chemotherapy in patients with Ki-67 < 55% (RR:  
250 15%) compared to patients with a Ki-67  $\geq$  55% (RR: 42%) (Sorbye et al. 2013). Data for advanced

NET G3 are generally scarce; however, RR to platinum-based chemotherapy is low (0-17%) with a short PFS (2.4 months)(Sorbye et al. 2018). Median survival is reported to be more than 40 months but as data is presented as a mixture of stages, results are difficult to interpret (Heetfeld et al. 2015; Hijioka, et al. 2017; Sorbye et al. 2018; Velayoudom-Cephise et al. 2013). In a high-grade GEP-NEN population of 136 patients, median survival from time of first diagnosis was best for NET G3 (43.6 months), intermediate for NEC with a Ki-67 21-54% (24.5 months) and 5.3 months for NEC cases with a Ki-67  $\geq 55\%$  (Milione et al. 2017). A combination of capecitabine and temozolomide has been suggested for patients with well differentiated tumor morphology and a Ki-67 21-54%, but data are scarce (Garcia-Carbonero et al. 2016; Heetfeld et al. 2015; Sorbye et al. 2018). In our cohort half the patients were treated with somatostatin analogs (SSA) either before and/or after PRRT. SSA is not recommended for high-grade NEN, but may be explained by the selection of patients with a positive SRI or use of SSA after PRRT in general.

Cross-trial comparisons are difficult as well as evaluation of the benefit of PRRT without a control arm. However, a RR of 42% and DCR of 69% indicate that PRRT has an effect in our cohort. No differences in RR were observed in subgroups according to both well vs. poor differentiation and Ki-67 21-54% vs. Ki-67  $\geq 55\%$ , as RR was approximately 40% in all subgroups. It may be that the efficacy of PRRT mediated by radiation is less sensitive to the degree of differentiation and rate of proliferation as long as the somatostatin receptor target is present on the tumor cells. The benefit of platinum based chemotherapy seems to be more dependent on a high degree of proliferation, as evident in the Nordic NEC study (Sorbye et al. 2013). As most of our patients had radiologically progressive disease at the start of PRRT, a PFS of 14 months indicates that PRRT seems to benefit many patients. Interestingly, no differences in RR, PFS and OS were evident in our cohort in regard to the line of treatment. Differentiation, Ki-67, PS, LDH and ALP were all significantly correlated to OS, as shown in previous studies (Lamarca, et al. 2017; Sorbye et al. 2013). However, the true

benefit of PRRT for PFS and especially OS is not possible to decide without a prospective randomized trial, which will be difficult to perform in such a rare disease. As implementation of PRRT may seem more likely in NET G3, such a randomized trial could compare PRRT vs standard chemotherapy regime (platinum-based or temozolomide/capecitabine) in a GEP NET G3 population. Data to clarify whether concurrent chemotherapy to PRRT should be considered is awaited (ClinicalTrials.gov: NCT02736448). Safety of PRCRT has been reported for 65 patients with 5-year follow-up, showing modest reversible hematological toxicity and comparable to PRRT (Kesavan, et al. 2014).

#### **Comparison with previous PRRT data in NEN G3 and classification**

Three single-center retrospective studies recently reported the outcome of PRRT in NEN with a high Ki-67 and SRI tumor uptake > liver.

An Australian study (Thang et al. 2018) assessed 28 patients with NEN and Ki-67 > 20% (median Ki-67: 32.5%). The majority received PRRT with concurrent chemotherapy. The RR was 35%, PFS 9 months and OS 19 months for all patients. According to Ki-67 index PFS (12 vs. 4 months) and OS (46 vs. 7 months) differed for Ki-67  $\leq$ 55% and Ki-67 > 55%.

A German study (Zhang, et al. 2018) assessed 69 patients with GEP NEN and Ki-67 index >20 % (median Ki-67 30 %). In their study, approximately one third received concurrent chemotherapy – the effect hereof was uncertain. The RR was 31 %, DCR 78 %, PFS 10 months and OS 20 months (rounded values). According to Ki-67 index PFS (11 vs. 4 months) and OS (22 vs. 7 months) differed for Ki-67  $\leq$ 55% and Ki-67 > 55%.

An Italian study (Nicolini, et al. 2018) assessed 33 patients with GEP NEN and Ki-67 index of 15-70% (median Ki-67: 25%). The RR was 6%, PFS 23 months and OS 52.9 months.

298 Overall, in our study we found similar results: PFS (16 vs.6 months) and OS (31 vs.9 months)  
299 differed significantly in patients with Ki-67 < 55% vs. Ki-67 ≥ 55%.

300 In general, the likelihood of somatostatin receptor expression on neuroendocrine cells  
301 decreases with increasing grade of tumor, whereas the opposite applies for FDG uptake (Binderup,  
302 et al. 2010; Hicks, et al. 2017). NET G3 seems to have a positive SRI uptake in 70% of cases,  
303 whereas for NEC the figure is more likely 30% (Heetfeld et al. 2015; Raj et al. 2017; Sorbye et al.  
304 2018; Sorbye et al. 2013; Velayoudom-Cephise et al. 2013). Preliminary studies have also shown  
305 the effectiveness of PRRT in patients with a more aggressive grade NEN with <sup>18</sup>F-FDG-avid and  
306 SRI uptake (Kashyap, et al. 2015). Patients with concordant <sup>18</sup>F-FDG and SRI avid lesions may be  
307 more radiosensitive by having a high proliferative fraction. Few of the patients in our cohort had  
308 <sup>18</sup>F-FDG PET/CT data available limiting further analysis.

309 As previously reported (Basturk, et al. 2015), the grading of NEN according to Ki-67  
310 may be optimized by further subclassification of patients with Ki-67 > 20%. In the current study of  
311 patients graded as NEN G3 based on Ki-67, nearly half the patients had well-differentiated tumor  
312 morphology. The majority of patients with well-differentiated tumors also had Ki-67 21-54%. There  
313 was a marked difference in outcomes in our cohort when comparing subgroups based on tumor  
314 morphology: PFS (19 vs.8 months) and OS (44 vs.19 months) differed significantly comparing  
315 well differentiated vs. poorly-differentiated neoplasms.

316

## 317 **Toxicity**

318 In our study, 26 patients (17%) had either acute or long-term grade 3-4 renal or hematological  
319 toxicity. This is similar to that reported in other larger retrospective analysis of patient groups given  
320 PRRT (Imhof et al. 2011; Kwekkeboom et al. 2008), although in NETTER-1 no evidence of renal



adverse effects was observed in patients treated with  $^{177}\text{Lu}$  (Strosberg et al. 2017). We observed renal toxicity both in patients treated with  $^{90}\text{Y}$  and  $^{177}\text{Lu}$ . Furthermore, we found similar frequency of toxicity for patients receiving PRRT as first line vs. later line of treatment.

## **Limitations**

High-grade GEP NEN patients treated with PRRT are probably highly selected on factors as being positive on SRI imaging and having a rather low median Ki-67 compared to the NEN G3 group as a whole. RR, PFS and OS should be interpreted carefully in light of the retrospective design of the study. However most of our patients were classified as having radiological progression of disease at the start of PRRT, and approximately half were based on RECIST. The rate of side-effects of PRRT in our analysis was in line with that previously reported for PRRT, but toxicity reports in a retrospective study must be interpreted cautiously. Pathologist reports were mainly from NET expert centers and reclassification was done in reports with missing data when sections were available. Though, a general problem is that the distinction between well and poor differentiation is not standardized (Tang, et al. 2016). Addition of molecular data on DAXX, ATRX (loss of expression in well-differentiated tumors) and Rb1, KRAS and p53 (expressed in poorly-differentiated tumors, could aid further in classification of differentiation (Sorbye et al. 2018).

## **Conclusion**

This large retrospective multicenter study is at present the most comprehensive report on which to base treatment decisions regarding the use of PRRT in high-grade GEP NEN. It shows promising RR, DCR, PFS and OS and acceptable toxicity after PRRT in patients with mainly progressive

343 disease. This suggests that PRRT is active and potentially effective in patients with GEP NEN G3.  
344 Awaiting further data, PRRT may therefore be a treatment option for GEP NEN G3 patients.

345

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485

**Table 1. Baseline characteristics of 149 patients with GEP NEN G3 receiving PRRT.**

Characteristics	Value
Age (years), median (range)	57 (24-85)
Time since diagnosis (m), median (range)	8 (0-174)
Gender, n (%): Male	76 (51.0)
Female	73 (49.0)
PS, n (%): 0	74 (49.7)
1	41 (27.5)
2	11 (7.4)
Missing	23 (15.4)
Primary tumor site, n (%): Pancreas	89 (59.7)
Gastrointestinal	34 (22.8)
Unknown primary	26 (17.4)
Metastatic disease, n (%)	147 (98.7)
Liver metastases	141 (94.6)
Tumor differentiation, n (%): Well	60 (40.3)
Intermediate	9 (6.0)
Poor	62 (41.6)
Not specified	18 (12.1)
Ki-67 (%), median (range)	30 (21-100)
21-54%	125 (83.9)
≥ 55%	23 (15.4)
Not specified	1 (0.7)
Ki-67 and differentiation: NET G3	58 (38.9)
NEC; Ki-67 21-54%	44 (29.5)
NEC; Ki-67 ≥ 55%	17 (11.4)
Not specified	30 (20.1)
CgA staining of tumor: Strongly positive	90 (60.4)
Partly positive	19 (12.8)
Negative	9 (6.0)
Not specified	31 (20.8) *
Synaptophysin staining of tumor: Strongly positive	105 (70.5)
Partly positive	11 (7.4)
Not specified	33 (22.1)
SRI available	146 (98.0%)
Uptake: None	0
< liver	5 (3.4%)
= liver	10 (6.7%)
> liver	131 (87.9%)
<sup>18</sup> F-FDG PET/CT available	39 (26.2%)
Tumor positive	34 (87.2%)
Plasma-CgA (n, %): Normal	15 (10.1)
Elevated	83 (55.7)
Missing	51 (34.2)
Plasma-LDH, n(%): Normal	76 (51.0)
Elevated	35 (23.5)
Missing	38 (25.5)
Plasma-ALP, n (%): Normal	54 (36.2)
Elevated	67 (45.0)
Missing	28 (18.8)
Number of prior lines of medical treatment: 0	30 (20.1)
1	62 (41.6)
2	31 (20.8)
> 2	26 (17.5)
Prior treatment, n (%)	

Primary tumor resected	58 (38.9)
Somatostatin analog	74 (49.7)**
Chemotherapy/targeted therapy	88 (59.1)
Cisplatin	31 (20.8)
Carboplatin	26 (17.4)
Etoposide	46 (30.9)
Capecitabine or 5-fluorouracil	38 (25.5)
Temozolomide	19 (12.8)
Streptozotocin	13 (8.7)
Everolimus	9 (6.0)
Doxorubicin	5 (3.4)
Sunitinib	4 (2.7)
Oxaliplatin	4 (2.7)
Interferon	2 (1.3)

487 PS: Performance status. CgA: chromogranin A. SRI: Somatostatin receptor imaging. LDH: lactate dehydrogenase.

488 ALP: alkaline phosphatase.

489 \* In 29 patients, CgA and synaptophysin staining results were not available; hereof 28 patients had SRI available that  
490 showed tumor uptake.

491 \*\*Missing values for seven patients.

492



493 **Table 2. Treatment details and toxicity of PRRT for 149 patients with GEP NEN G3.**

	Value
Radiologically progressive disease at start of PRRT, n (%)	104 (69.8)
No	35 (23.5)
Unknown	10 (6.7)
Indication for PRRT, n (%)	
Progression of disease	97 (65.1)
First line	30 (20.1)
Side effects to other therapies	6 (4.0)
Other	16 (10.7)
Radioisotope, n (%)	
<sup>177</sup> Lutetium	101 (67.8)
<sup>90</sup> Yttrium	34 (22.8)
<sup>177</sup> Lutetium + <sup>90</sup> Yttrium	12 (8.1)
<sup>111</sup> Indium	1 (0.7)
Not specified	1 (0.7)
Cumulative activity (GBq), median (range)	18.0 (4-85)
Number of PRRT cycles, median (range)	4.0 (1-15)
Fulfilled planned number of cycles	98 (65.8)
Discontinuation of PRRT:	
Disease progression	19 (12.8)
Clinical deterioration	6 (4.0)
Hematological side effects	5 (3.4)
Renal side effects	1 (0.7)
Lack of compliance	1 (0.7)
Other	17 (11.4)
Not specified	2 (1.3)
WHO performance status after treatment, n (%)	
0	74 (49.7)
1	34 (22.8)
2	11 (7.4)
3	5 (3.4)
Not specified	25 (16.8)
Absence of acute toxicity (grade 3-4), n (%)	121 (81.2)
Acute toxicity	19 (12.8)
Hematological, grade 3/grade 4, n *	8/1
Renal	2/1
Diarrhea	0/2
Nausea	0/2
Other, not specified	14/1
Unknown	9 (6.0)
Absence of long-term toxicity (grade 3-4), n (%)	101 (67.8)
Long-term toxicity	19 (12.8)
Hematological, grade 3/grade 4, n *	13/2
Renal	3/0
Other, not specified	3/3
Unknown	29 (19.5)

494 \*More than one may be reported for a patient.

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498 **Table 3. PRRT response rates (n=114) and outcomes (n=149) in GEP NEN G3.**

	<b>CR (%)</b>	<b>PR (%)</b>	<b>SD (%)</b>	<b>PD (%)</b>	<b>PFS (m), (95% CI)</b>	<b>OS (m), (95% CI)</b>	
All patients	1 (1)	47 (41)	43 (38)	23 (20)	14.0 (10.4-17.6)	29.0 (23.3-34.7)	
PS							
• 0	1 (2)	21 (36)	26 (45)	10 (17)	<b>16.0 (11.0-21.0)</b>	<b>39.0 (28.1-49.9)</b>	*
• 1	0	17 (53)	8 (25)	7 (22)	<b>14.0 (8.2-19.8)</b>	<b>23.0 (16.2-29.8)</b>	
• 2	0	3 (38)	2 (25)	3 (38)	<b>3.0 (0-6.2)</b>	<b>4.0 (0-12.6)</b>	
SRI tumor uptake							
• ≤liver	1 (9)	3 (27)	4 (36)	3 (27)	16.0 (7.9-24.1)	25.0 (8.6-41.4)	
• > liver	0	44 (43)	38 (37)	20 (20)	14.0 (10.0-18.0)	29.0 (21.6-36.4)	
Primary tumor site							
• Pancreas	0	32 (48)	23 (34)	12 (18)	14.0 (10.4-17.6)	29.0 (21.7-36.3)	
• Gastrointestinal	0	11 (42)	9 (35)	6 (23)	10.0 (0-21.2)	31.0 (7.5-54.5)	
• Unknown	1 (5)	4 (19)	11 (52)	5 (24)	16.0 (8.4-23.6)	29.0 (11.4-46.6)	
Differentiation							
• Well	0	19 (42)	23 (51)	3 (7)	<b>19.0 (13.9-24.1)</b>	<b>44.0 (25.2-62.8)</b>	*
• Poor	1 (2)	21 (41)	13 (25)	16 (31)	<b>8.0 (3.3-12.7)</b>	<b>19.0 (11.7-26.3)</b>	
Proliferation							
• Ki-67 21-54%	1 (1)	41 (41)	41 (41)	16 (16)	<b>16.0 (12.7-19.3)</b>	<b>31.0 (24.2-37.8)</b>	*
• Ki-67 ≥ 55%	0	6 (43)	2 (14)	6 (43)	<b>6.0 (3.0-9.0)</b>	<b>9.0 (4.5-13.5)</b>	
Differentiation and proliferation							
• NET G3	0	18 (42)	22 (51)	3 (7)	<b>19.0 (14.4-23.6)</b>	<b>44 (25.3-62.7)</b>	*
• NEC; Ki-67 21-54%	1 (3)	16 (41)	12 (31)	10 (26)	<b>11 (5.4-16.6)</b>	<b>22.0 (16.0-28.0)</b>	
• NEC; Ki-67 ≥ 55%	0	5 (45)	1 (9)	5 (45)	<b>4 (0.8-7.2)</b>	<b>9.0 (1.6-16.4)</b>	
Line of treatment							
• First line	0	10 (42)	9 (38)	5 (21)	13 (6.3-19.7)	29 (12.5-45.5)	
• Second line	0	20 (45)	16 (36)	8 (18)	12.0 (6.5-17.5)	29 (16.8-41.2)	
• Later line	1 (2)	17 (37)	18 (39)	10 (22)	19.0 (13.6-24.4)	29.0 (18.0-40.0)	
plasma-LDH							
• Normal	1 (2)	30 (48)	24 (38)	8 (13)	<b>18.0 (14.3-21.7)</b>	<b>39.0 (30.8-47.2)</b>	*
• Elevated	0	8 (30)	8 (30)	11 (41)	<b>4.0 (0.5-7.5)</b>	<b>13.0 (7.4-18.6)</b>	
plasma-ALP							
• Normal	1 (2)	21 (47)	18 (40)	5 (11)	<b>18.0 (9.9-26.1)</b>	<b>39.0 (30.8-47.2)</b>	*
• Elevated	0	22 (40)	19 (35)	14 (25)	<b>14.0 (9.2-18.8)</b>	<b>21.0 (16.8-25.2)</b>	

\*Denotes statistically significant difference in PFS and OS. P-values as shown in Figures 1-2.

CR: complete response. PR: partial response. SD: stable disease. PD: progressive disease per Response Evaluation Criteria In Solid Tumours. PFS: progression-free survival. OS: overall survival. CI: confidence interval. SRI: somatostatin receptor imaging. LDH: lactate dehydrogenase. ALP: alkaline phosphatase.

510 **Table 4. Multiple Cox regression analysis of predictors for PFS and OS in 75 GEP NEN G3**  
511 **patients treated with PRRT. (74 missing)**

<b>Model 1</b>	<b>PFS</b>		<b>OS</b>	
<b>Covariate</b>	<b>Hazard ratio (95% CI)</b>	<b>P-value</b>	<b>Hazard ratio (95% CI)</b>	<b>P-value</b>
<i>Age</i>	<b>0.98 (0.95-1.00)</b>	<b>0.045</b>	0.99 (0.96-1.02)	0.42
<i>Male</i>	1.53 (0.85-2.74)	0.16	1.05 (0.53-2.10)	0.89
<i>PS 0</i>	1		1	
1	<b>2.57 (1.33-4.93)</b>	<b>0.005</b>	<b>2.35 (1.13-4.89)</b>	<b>0.02</b>
2	3.42 (0.90-13.06)	0.07	<b>4.20 (0.98-18.01)</b>	<b>0.05</b>
<i>SRI ≤ liver</i>	0.72 (0.13-4.03)	0.70	0.43 (0.04-4.33)	0.47
<i>Primary tumor site (unknown primary)</i>	1		1	
Gastrointestinal	0.80 (0.32-2.02)	0.64	0.78 (0.26-2.37)	0.66
Pancreas	0.66 (0.28-1.57)	0.35	0.46 (0.18-1.22)	0.12
<i>Poorly differentiated</i>	1.69 (0.88-3.23)	0.11	<b>2.92 (1.31-6.50)</b>	<b>0.009</b>
<i>Ki-67 ≥ 55%</i>	1.11(0.51-2.42)	0.80	1.97 (0.83-4.66)	0.13
<i>Line of treatment (first line)</i>	1		1	
Second line	0.76 (0.38-1.54)	0.46	1.55 (0.70-3.43)	0.28
Later line	1.04 (0.48-2.27)	0.91	1.77 (0.67-4.65)	0.25
<i>Elevated plasma-LDH</i>	<b>2.66 (1.29-5.49)</b>	<b>0.008</b>	<b>2.61 (1.16-5.90)</b>	<b>0.02</b>
<i>Elevated plasma-ALP</i>	<b>2.24 (1.22-4.09)</b>	<b>0.009</b>	<b>2.79 (1.42-5.49)</b>	<b>0.003</b>

522 **Supplementary Table 1. Univariate analyses of predictors for PFS and OS in 149 GEP NEN**  
523 **G3 patients treated with PRRT.**

Covariate	PFS		OS	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
<i>Age</i>	1.00 (0.99-1.02)	0.84	1.01 (0.99-1.03)	0.20
<i>Male</i>	0.96 (0.68-1.34)	0.79	0.78 (0.53-1.1)	0.19
<i>PS 0</i>	1		1	
1	1.36 (0.91-2.04)	0.14	<b>1.65 (1.04-2.63)</b>	<b>0.04</b>
2	<b>3.53 (1.83-6.83)</b>	<b>&lt; 0.001</b>	<b>6.84 (3.40-13.76)</b>	<b>&lt; 0.001</b>
<i>SRI ≤ liver</i>	1.17 (0.67-2.04)	0.59	0.79 (0.40-1.57)	0.50
<i>Primary tumor site (unknown primary)</i>	1		1	
Gastrointestinal	1.13 (0.65-1.95)	0.67	0.75 (0.41-1.39)	0.36
Pancreas	1.29 (0.80-2.07)	0.30	0.83 (0.50-1.37)	0.46
<i>Poorly differentiated</i>	<b>1.62 (1.11-2.36)</b>	<b>0.01</b>	<b>2.55 (1.62-4.02)</b>	<b>&lt; 0.001</b>
<i>Ki-67 ≥ 55%</i>	<b>2.15 (1.34-3.47)</b>	<b>0.002</b>	<b>2.48 (1.51-4.06)</b>	<b>&lt; 0.001</b>
<i>Differentiation and proliferation (NET G3)</i>	1		1	
NEC; Ki-67 21-54%	1.38 (0.91-2.07)	0.13	<b>2.06 (1.26-3.39)</b>	<b>0.004</b>
NEC; Ki-67 ≥ 55%	<b>2.81 (1.55-5.11)</b>	<b>0.001</b>	<b>4.77 (2.51-9.06)</b>	<b>&lt; 0.001</b>
<i>Line of treatment (first line)</i>	1		1	
Second line	1.08 (0.69-1.69)	0.73	1.04 (0.63-1.71)	0.87
Later line	0.79 (0.50-1.24)	0.31	0.86 (0.52-1.42)	0.55
<i>Elevated plasma-LDH</i>	<b>2.35 (1.54-3.59)</b>	<b>&lt; 0.001</b>	<b>3.14 (1.96-5.02)</b>	<b>&lt; 0.001</b>
<i>Elevated plasma-ALP</i>	<b>1.53 (1.04-2.24)</b>	<b>0.03</b>	<b>2.21 (1.42-3.45)</b>	<b>&lt; 0.001</b>

524 CI: Confidence interval. PS: performance status. SRI: Somatostatin receptor imaging. GI: gastrointestinal. LDH: lactate  
525 dehydrogenase. ALP: alkaline phosphatase.

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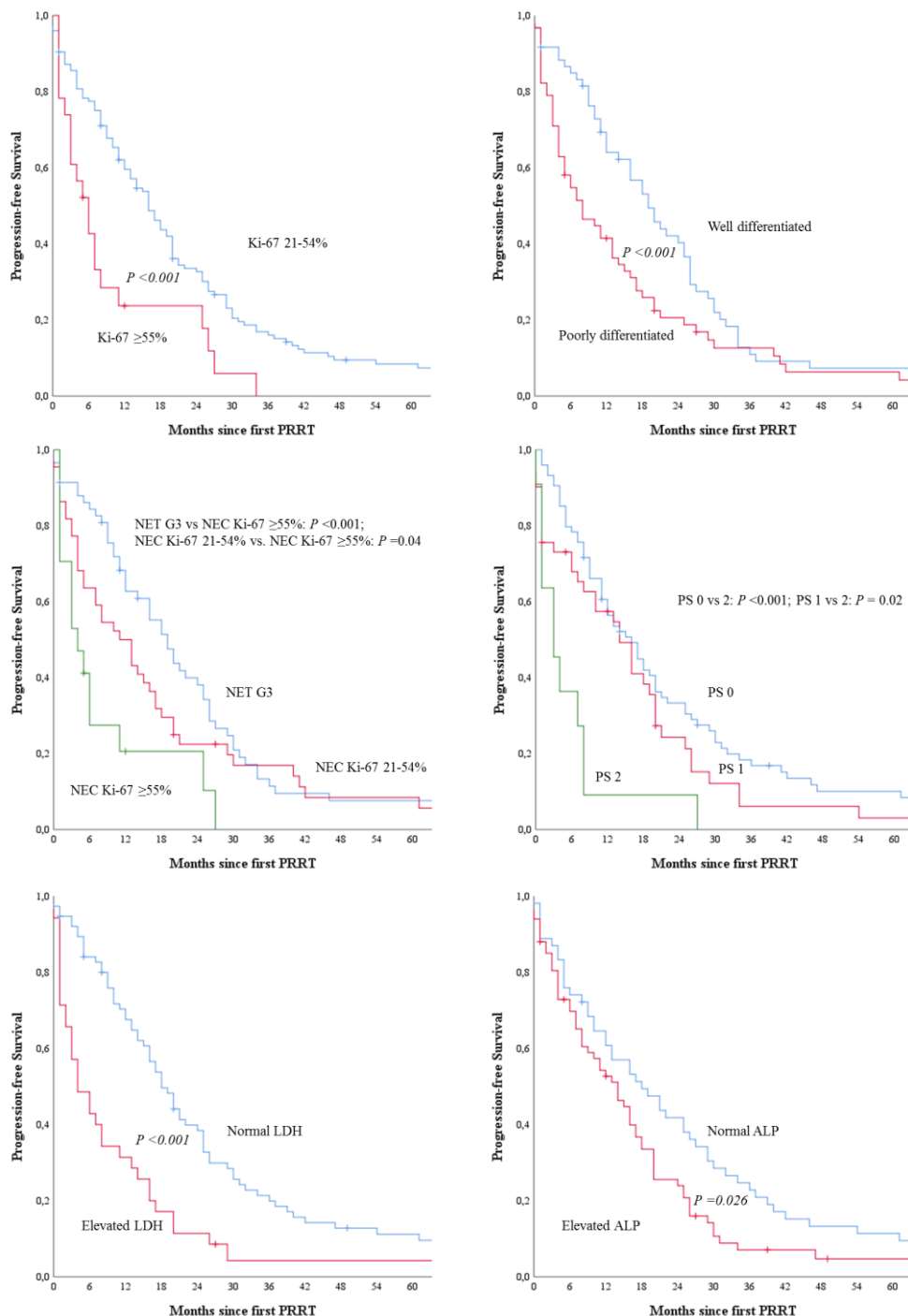
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532 **Supplementary table 2. Multiple Cox regression analysis of predictors for PFS and OS in 106**  
533 **GEP NEN G3 patients treated with PRRT. (43 missing)**

<b>Model 2</b>	<b>PFS</b>		<b>OS</b>	
<b>Covariate</b>	<b>Hazard ratio (95% CI)</b>	<b>P-value</b>	<b>Hazard ratio (95% CI)</b>	<b>P-value</b>
<i>Age</i>	1.00 (0.99-1.02)	0.66	1.02 (0.99-1.04)	0.16
<i>Male</i>	1.13 (0.71-1.80)	0.60	0.86 (0.48-1.54)	0.62
<i>PS 0</i>	1		1	
1	<b>1.74 (1.07-2.86)</b>	<b>0.03</b>	<b>1.86 (1.07-3.24)</b>	<b>0.03</b>
2	<b>4.10 (1.82-9.24)</b>	<b>&lt; 0.001</b>	<b>5.39 (2.15-13.52)</b>	<b>&lt; 0.001</b>
<i>SRI ≤ liver</i>	0.86 (0.33-2.21)	0.75	0.16 (0.02-1.20)	0.07
<i>Primary tumor site (unknown primary)</i>	1		1	
Gastrointestinal	1.01 (0.50-2.01)	0.98	0.51 (0.23-1.11)	0.09
Pancreas	1.16 (0.63-2.12)	0.63	0.62 (0.32-1.19)	0.15
<i>Poorly differentiated</i>	<b>1.84 (1.16-2.94)</b>	<b>0.01</b>	<b>3.16 (1.73-5.76)</b>	<b>&lt; 0.001</b>
<i>Ki-67 ≥ 55%</i>	1.30 (0.68-2.48)	0.43	1.69 (0.81-3.52)	0.16
<i>Line of treatment (first line)</i>	1		1	0.19
Second line	1.15 (0.64-2.07)	0.63	1.55 (0.78-3.10)	0.21
Later line	0.75 (0.40-1.43)	0.39	0.98 (0.45-2.13)	0.95

**Figure 1. Kaplan-Meier curves of PFS for 149 patients with GEP NEN G3 treated with PRRT. Stratification by Ki-67 index (n=148), differentiation (n=122), performance status (n=126), combined Ki-67 index and differentiation (n=119), LDH (n=111) and ALP (n=121), respectively. P-values shown in figures for statistically significant differences.**



571 **Figure 2. Kaplan-Meier analysis of OS for 149 patients with GEP NEN G3 treated with**  
 572 **PRRT. Stratification by Ki-67 index (n=148), differentiation (n=122), performance status**  
 573 **(n=126), combined Ki-67 index and differentiation (n=119), LDH (n=111) and ALP (n=121),**  
 574 **respectively. P-values shown in figures for statistically significant differences.**

